

**REMARKS**

Amendments to the claims have been made to refine claim language. In making the amendments, it should be understood that the amendments are to be interpreted in a manner that avoids, where applicable, any admission or estoppel, generally, negatively affecting the scope of protection provided by the disclosure and claims of the present application, and also in a manner that avoids prosecution history estoppel, limitation of the scope of equivalences, or the like.

As to the issues raised by the examiner, it should be understood that support does exist in the specification for the current and previous amendments. As to the current amendments, support concerns are addressed by highlighting areas in specification supporting the following claim aspects:

In claim 63 as amended, the aspect of: "equilibrating at least some of said sex-selected sperm cells suspended in said extender to a cooler, non-freezing temperature for a period of time prior to freezing over a period of about 1 hour to about 18 hours" is explicitly discussed in paragraph 76 (with numbering as indicated in the publication of the application), as shown on page 17, line 5 of the original specification where it is stated:

Prior to freezing, the sperm are generally allowed to equilibrate at about 5.degree. C. Preferably, the sperm are allowed to equilibrate for a period in the range of **about 1 hour to about 18 hours**, more preferably between about 3 hours and about 18 hours, and most preferably between about 3 hours and about 6 hours (see Example 2). (Emphasis added.)

From this excerpt, it is clear that the time limitation now posed, namely, the "period of about 1 hour to about 18 hours" is not new matter and was originally discussed in the application.

In claim 64 as amended, the aspect of: "equilibrating at least some of said sex-selected sperm cells suspended in said extender to a cooler, non-freezing temperature for a period of time prior to freezing over a period of not greater than 6 hours" is also addressed in the specification. First, paragraph 76 (quoted above) of the original specification discusses the aspect of equilibrating for between about 3 hours and about 6 hours. Table 4 also shows data that a person of ordinary skill in the art would readily understand as demonstrating that equilibration for a period of not greater than six hours yields higher motility percentages. Specifically, reviewing each of the 0h, 1h, and 2h post

thaw assessment times shown in the table, it is evident that motility has a significant drop when equilibration occurs for more than six hours. In the 0h situation motility dropped by 15% (35% motile vs. 41% motile for the shorter equilibrations times). Using the 1h time of assessment, motility similarly dropped by 16% (31% motile vs. 37% motile for the shorter equilibration time). Further, even using the 2h time of assessment, motility dropped by a significant 33% (12% motile vs. 18% motile for the shorter equilibration time). In addition to this data, there is additional discussed in paragraph 114 and 115 (again, with numbering as indicated in the publication of the application), as shown on page 27, line 16 of the original specification where it is stated:

6. Conclusion. The results indicated no differences in post-thaw sperm motility between 3 and 6 h of total equilibration time at 5.degree. C., but there was a significant decline in sperm motility following 18 h of equilibration at 5.degree. C. before freezing. The 3- to 6-h range permits pooling 2 consecutive 3-h sorting batches for freezing sperm without decreasing post-thaw motility.

As the bull by equilibration-time interaction was not significant, 3 to 6 h equilibration was adequate, with the caveat that only 4 bulls were used. The optimum equilibration time for a minority of bulls is expected to be >6 h.

This excerpt makes it clear that only a "minority of bulls" would be expected to have an optimum equilibration time of "greater than 6 hours". From this, a person of ordinary skill in the art would easily understand that, therefore, the majority of bulls are expected to have a equilibration time of "not greater than 6 hours" as now set forth in the claim. Thus it is clear that the time limitation now posed, namely, the period of "not greater than 6 hours" is not new matter and is supported by the original application.

In claim 66, as amended, the aspect of: "suspending at least some of said sperm cells in said extender results in a final concentration of said sex-selected sperm cells of greater than 10 million per milliliter of extender" is explicitly discussed in paragraph 88 (with numbering as indicated in the publication of the application), as shown on page 20, line 9 of the original specification where it is stated:

5. Results. Data for non-washed sperm (Table 1) revealed (log) linear relationships ( $P<0.01$ ) for both incubation times. Percentages of motile sperm increased as sperm concentration increased from  $1.25 \times 10^6/\text{ml}$  to  $10 \times 10^6/\text{ml}$ , but there was little difference thereafter. The cubic term was significant ( $P<0.05$ ) for 24-h and marginally significant ( $P<0.1$ ) for 48-h incubations. There was a bull effect ( $P<0.01$ ) at both times. (Emphasis added.)

Table 1 as shown on page 22, also shows data that would indicate to a person of ordinary skill in the art that concentrations of greater than 10 million per milliliter are significant to achieving higher motility percentages. Specifically, reviewing the data, it is evident that motility has a significant drop when concentrations are less than 10 million per milliliter. Most notably, in the 48h incubation situation, motility drops by 26% when concentration is below 10 million per milliliter (31% motile vs. 42% motile for the lower concentration value). From these disclosures in the original specification, it is clear that the concentration limit now posed, namely, the "greater than 10 million per milliliter" concentration value is not new matter and was originally discussed in the application. [Note that the earlier 15 million/ml limit is also supported in the original specification, however, this limit is not desired as the claim limitation at this time.]

In addition to the request for the location of the discussions mentioned above for new matter concerns, the action also raised clarity concerns. Each of these has been addressed by amending the language to clarify that which is intended. Specifically, claim 38 has been amended to remove the term "male" as questioned, to resolve the antecedent basis of "sex-selected sperm cells", and to eliminate the potentially confusing "portion" language. Similarly, the questioning of the term "equilibrate" has been clarified in claims 63 and 64, to indicate that the equilibration is to a cooler, non-freezing temperature for a period of time.

Finally, as to the obviousness concerns, this application has been amended to specify the particular type of sorting is the type using flow cytometry. This particular type of sorting is significant because one would usually prefer a sorting method that preserves sperm viability. The challenging conditions (for sperm) such as exist in flow cytometry sorting would usually be avoided, but instead through this invention, these conditions can be overcome. Specifically, the original application beginning in paragraph 38 (numbering as indicated in the publication of the application) shown on page 6, line 20, states:

The selection method used to obtain the selected sperm sample is preferably one that preserves sperm viability. Because of the relative fragility of sperm, normal flow cytometry techniques should generally be modified for sorting sperm. More specifically, the flow cytometry entails staining, dilution, and interrogation of cells with light. All of these steps represent stresses that can reduce sperm viability. The

sensitivity of sperm to these stresses can vary between species and even between individuals within species. Such sensitivities have either been documented or can readily be determined by empirical studies, such as those described in Examples 1-5.

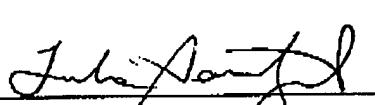
This application highlights the challenges of using flow cytometry for the separation task and also highlights how the combination of freeing with flow cytometry sorting has been overcome. Importantly, none of the references discuss the unique combination of flow cytometry with a freezing process as now possible. Thus it is respectfully submitted that the claims as amended are not obvious in view of the cited references. Finally, as to this and all amendments, it should be understood that this amendment is made without prejudice and with the express reservation of the right to pursue claims without this limitation in this or other applications.

### CONCLUSION

The claims have been amended to address matters of form as requested in the action. Claims 40-41, 55-56, 60-62, 65, and 67 have been withdrawn. Claims 38-39, 42-54, 57-59, 63-64, and 66 remain in the application. An early consideration of the present application is earnestly requested.

Dated this 17<sup>th</sup> day of October, 2003.

Respectfully submitted,

  
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